



## Quantification of Inkjet-Printed Pharmaceuticals on Porous Substrates Using Raman Spectroscopy and Near-Infrared Spectroscopy

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## 1 Introduction

2 The use of inkjet printing (IJP) technology is gaining considerable interest for additive manufacturing of  
3 pharmaceutical drug delivery systems (1, 2). Printing offers multiple advantages for manufacturing of drug  
4 products such as precise dosing, production of multi-dose regimens and ultimately personalized medicine,  
5 optimized for the treatment of the specific patient (3). When conducting IJP of pharmaceuticals, the active  
6 pharmaceutical ingredient (API) is either dissolved or suspended in an ink base and then precisely  
7 deposited on the substrate, i.e., a carrier, from a printer's nozzle in a digitally controlled way (4). In spite of  
8 the evident benefits of IJP, production challenges exist in regard to using inkjet printing for manufacturing  
9 of personalized medicine. Inkjet printing of pharmaceuticals is currently a low-output method, meaning  
10 that the number of dosage forms produced in IJP is limited. While it is easy to conduct API content analysis  
11 using well-established methods such as UV spectroscopy and HPLC analysis, these methods require the use  
12 of destructive sample preparation that is costly. Therefore, the API content analysis of the printed dosage  
13 forms should ideally be conducted using non-destructive, robust and fast methods (5). This is especially  
14 important in the future to realize on-demand and near the end-users pharmacoprinting of personalized  
15 medicine (6) or to integrate it in a continuous manufacturing setup (7, 8) according to the emerging  
16 regulatory framework (9). Recently, multiple methods have been described for non-destructive API content  
17 analysis in printed dosage forms. Vakili et al. used near-infrared chemical imaging for content analysis of IJP  
18 theophylline using copy paper as the model substrate (10). Researchers from the same group have used a  
19 colorimetric technique for content determination of IJP propranolol with colorant added, using edible rice  
20 paper and edible icing sheets as the substrates (11) and was also used for quantitative determination of IJP  
21 vitamin B2 doses on edible rice paper and copy paper (12). More recently, a handheld near-infrared  
22 spectrometer has been reported for API quantification on the IJP dosage forms, containing levothyroxine  
23 sodium and prednisolone. Edible icing sheets and solvent-casted films, containing cellulose derivatives,  
24 were used as the substrates (13). ATR-FTIR was used for quantifying loperamide and caffeine printed on  
25 poly-tetrafluoroethylene films (14). Recently, our group used Raman spectroscopy for the quantification of  
26 IJP haloperidol on inorganic compacts and commercial paracetamol tablets (15). Raman spectroscopy has  
27 also been used for the assessment of polymorphism of prednisolone IJP onto polytetrafluoroethylene  
28 (PTFE) fiber glass films (16). In addition, confocal Raman mapping was conducted to describe drug  
29 distribution in the multi-layered films with three jet-dispensed model drugs (17). While the analytical  
30 techniques mentioned here have the potential to be used in a manufacturing-on-demand setting (such as  
31 in a community pharmacy), they also have drawbacks. For example, the colorimetric technique determines  
32 the content of the printed API indirectly by quantifying the coloring agent, added to the ink along the API.

33 This could potentially lead to errors in estimating the actual API content due to, for example, degradation  
34 of the API, discoloration and/or migration of colorants. Measurements with spectroscopic techniques, such  
35 as near-infrared (NIR) and Raman spectroscopy, put certain demands on the morphology of the substrates,  
36 for instance, near-infrared chemical imaging requires the substrate to be completely flat in order to achieve  
37 accurate focusing of the diffusely reflected light (18). The handheld NIR method can in turn be prone to  
38 localized variation of the obtained spectra within the printed dosage form, e.g., if the substrate is porous  
39 and there is a variation in the absorption of the ink, or if the API crystallizes on the surface of the substrate,  
40 and therefore, different regions of the same printed area may give different results leading to errors in the  
41 correct determination of the drug content in the dosage form. Raman spectroscopy is also prone to  
42 variations in the material density of the substrate, e.g., when a highly porous vacuum-oven dried  
43 hypromellose was used as substrate, it was unsuitable for quantifying the drug content using spectroscopic  
44 methods (15). Besides that, measurements with NIR would be affected by residual solvents, in particular  
45 moisture from vapor sorption. It is evident from these examples that there is a need for gaining a better  
46 understanding about the analytical technique(s) in terms of its sensitivity and suitability to determine the  
47 drug content in a porous substrate.

48 In this study we describe inkjet printing of three APIs on novel porous substrates prepared from  
49 hypromellose and the investigation of two spectroscopic techniques for the non-destructive and accurate  
50 determination of the inkjet printed drugs. Three model APIs, namely montelukast, haloperidol and  
51 propranolol in various doses were printed on the custom-developed substrates. The printed doses were  
52 analyzed using NIR spectroscopy and Raman spectroscopy and the resulting spectra were correlated to the  
53 API content measured by HPLC using Partial Least Squares (PLS) regression. The resulting models were  
54 compared in regards to their accuracy and prediction power.

## 55 **Materials and Methods**

56 Three model active pharmaceutical ingredients (APIs) were used in this study: haloperidol and propranolol  
57 hydrochloride were purchased from Sigma Aldrich (St Louis, MI, USA), and montelukast sodium was  
58 obtained from Matrix Laboratories (Hyderabad, India). Propylene glycol (PG) and lactic acid (LA) were  
59 obtained from Sigma Aldrich, ethanol (96 %) was supplied by Altia OY (Helsinki, Finland). Hypromellose  
60 (hydroxypropyl methylcellulose), HPMC, Metolose® 60SH-4000, was purchased from Shin-Etsu Chemical  
61 Co. (Tokyo, Japan), macrogol 4000, polyethylene glycol 4000, PEG4000, and polysorbate 20, Tween® 20,  
62 were purchased from Fluka Analytical (Seelze, Germany). Erythrosine (E127) was supplied by Merck

63 (Darmstadt, Germany), blue food coloring liquid, containing brilliant blue (E133), was supplied by Dr.  
64 Oetker (Bielefeld, Germany).

#### 65 **Preparation of substrates**

66 A single substrate (sample name S3) was produced as described in a recent paper (19). It was prepared by  
67 mixing 5 g HPMC with 0.5 g macrogol 4000, glycerol and polysorbate 20 and 2.1 g poloxamer 188. This  
68 mixture was added to purified water at 70°C under stirring. The total mass of the mixture was 100 g. After  
69 cooling the mixture to room temperature, it was cast into several silicone molds (10×28 cm<sup>2</sup>). Casting was  
70 done so that the approximate height of the liquid in the mold was 5 mm. The formulation in the casting  
71 molds was cooled to 5 ± 3 °C for 24h in order to allow complete hydration of the polymer. The samples  
72 were then freeze-dried using an Epsilon 1-4 LSCplus Pilot Scale Freeze Dryer (Martin Christ, Osterode am  
73 Harz, Germany). The samples were cooled to -30°C over 3 h, then held isothermally for 3 h after which the  
74 pressure was reduced and kept at 0.12 mbar while the temperature was increased to 0°C over 16.5 h  
75 during primary drying.

#### 76 **Ink formulations**

77 Propranolol hydrochloride ink (50 mg/ml) was prepared by mixing PG:water in a ratio of 3:7. 10 drops of  
78 blue liquid fruit coloring were added through a cellulose pore filter (0.45 µm, Phenomenex, Torrance, CA,  
79 USA) to 100 ml of this mixture. 2.5 g propranolol hydrochloride was dissolved in this mixture in a 50.0 ml  
80 volumetric flask. The mixture was allowed to stand in an ultrasound bath for 30 min in order to ensure that  
81 API was dissolved.

82 Montelukast sodium ink (200 mg/ml) was prepared by mixing PG:ethanol in a 3:7 ratio in a 100 ml  
83 volumetric flask. Two grains (approx. 1 mg) of erythrosine were added to the mixture. 10.0 g montelukast  
84 sodium was added to the mixture in a 50.0 ml volumetric flask. The mixture was put in an ultrasound bath  
85 for 2 h in order to ensure that montelukast sodium is dissolved completely.

86 The haloperidol ink (160 mg/ml) was prepared by mixing LA:ethanol in a 16:84 ratio in a 50.0 ml volumetric  
87 flask. 1 drop of blue fruit coloring liquid and 1 grain of erythrosine was added resulting in slightly purple  
88 color. 4.0 g haloperidol was combined with the solvent mixture in a 25.0 ml volumetric flask. The mixture  
89 was put in the ultrasound bath for 30 min to dissolve haloperidol.

#### 90 **Inkjet printing**

91 Inkjet printing was done on a PiXDRO LP50 piezoelectric inkjet printer (Roth & Rau, Eindhoven,  
 92 Netherlands) mounted with a Spectra SL-128 AA print head with 128 nozzles (Fujifilm, Tokyo, Japan). The  
 93 ink for printing was loaded into the ink container through a syringe equipped with a cellulose pore filter  
 94 (0.45  $\mu\text{m}$ , Phenomenex®). Propranolol ink was printed using a voltage of 90 V and a pulse ratio of 90 % with  
 95 an ink pressure of -21.0 mbar. Montelukast ink was printed using a voltage of 120 V and a pulse ratio of  
 96 85% with an ink pressure of -22 mbar. The haloperidol ink was printed with a voltage of 120 V and a pulse  
 97 ratio of 90 % with a pressure of -23.9 mbar. All the inks were printed in  $1 \times 1 \text{ cm}^2$  squares and 6-8 samples  
 98 were printed for each dosing step. The number of layers printed and the dosage regimen for each API is  
 99 described in Table I. Drop shape and size analysis on all the inks were done using Advanced Drop  
 100 Calculation software (Meyer Burger Technologies, Eindhoven, Netherlands).

101 Table I. Overview of printed samples on the different substrates.

API	Number of layers printed	API content per step, mg	Dose regimen, mg (calculated)
Propranolol hydrochloride	5-35 in steps of 5	0.6	0.6; 1.2; 1.8; 2.4; 3.0; 3.5; 4.1
Montelukast sodium	5-30 in steps of 5	2.1	2.1; 4.2; 6.3; 8.4; 10.5; 12.6
Haloperidol	2-14 in steps of 2	0.6	0.6; 1.2; 1.8; 2.4; 3.0; 3.6; 4.2

102

### 103 **Dynamic viscosity and surface tension**

104 The viscosity of the API-containing inks was measured on an AR-G2 rheometer (TA Instruments, New  
 105 Castle, DE, USA), equipped with a cone-plate geometry ( $\varnothing = 60 \text{ mm}$ ). The cone angle was  $0.9811^\circ$ . 1.05 ml  
 106 of each ink solution was applied on the plate, thermostated to  $25 \text{ }^\circ\text{C}$ , and subjected to a stationary shear  
 107 stress ramp from 10 to  $1000 \text{ s}^{-1}$ . The average of three measurements of the viscosity at 10, 100 and  $1000 \text{ s}^{-1}$   
 108 was calculated and used to obtain the viscosity value of the ink by using Rheology Advantage software  
 109 v5.7.2 (TA Instruments).

110 The surface tension of the inks was measured with a DSA100 Drop Shape Analyzer (KRÜSS GmbH,  
 111 Hamburg, Germany). The data were analyzed using Drop Shape Analysis 1.90.0.22 software (KRÜSS).

### 112 **Microscopy**

113 Visible light microscopy was conducted on a DM LM microscopy (Leica Microsystems GmbH, Wetzlar,  
114 Germany) equipped with an Evolution MP Camera (Media Cybernetics, Rockville, MD, USA) controlled by  
115 Image-Pro Insight software v 8.0 (Media Cybernetics). The microscope was operated in both reflected-light  
116 mode and polarized-light mode using a 10X objective. The surface and cross-sections of the printed  
117 samples were imaged in reflected-light mode, taking multiple exposures, each focusing on a different part  
118 of the sample and the resulting images were stacked in Adobe Photoshop CC 2018 v 19.0.1 (Adobe Systems  
119 Inc, San Jose, CA, USA), using the Auto-Blend Layers Function.

#### 120 **High-performance liquid chromatography (HPLC)**

121 API quantification was performed on an Infinity 1260 HPLC system (Agilent Technologies, Santa Clara, CA,  
122 USA) using a C18 column (5  $\mu\text{m}$ , 150 mm  $\times$  4.6 mm). The HPLC system was controlled by Infinity 1260  
123 software (Agilent Technologies). The mobile phase for analysis of propranolol consisted of 0.067 mM  
124 phosphate buffer (pH 3) and acetonitrile in a 60:40 ratio. For montelukast, the mobile phase was 1 mM  
125 acetate buffer (pH 5.9):acetonitrile in a 10:90 ratio . Haloperidol mobile phase consisted of 50 mM  
126 phosphate buffer (pH 2.5):acetonitrile in a 75:25 ratio. Standard curves were done for all APIs and linearity  
127 was observed ( $R^2 = 0.998-0.999$ ).

#### 128 **Porosity and internal structure of the samples**

129 The porosity of the substrates was assessed using a custom-developed oil absorption method (20) and X-  
130 ray computed microtomography ( $\mu\text{CT}$ ). Samples of approximately 8 by 10 mm were cut out using a scalpel.  
131 The length, width and height of the samples were measured using a caliper (Mitutoyo Corporation,  
132 Kawasaki, Japan) and the mass was measured. The samples were then placed in paraffin oil ( $\rho = 0.862$ ) in a  
133 desiccator at a pressure of 40 mPa for 24 hours. Then the samples were removed and excess oil was wiped  
134 off using a tissue. The samples were then weighed and the porosity,  $\Phi$ , was calculated according to Eq. 1.

$$135 \quad \Phi = \frac{(M_o - M_s) / \rho_o}{V_s} \quad \text{Eq. 1}$$

136  $V_s$  is the volume of the sample,  $M_o$  is the sample weight after oil absorption,  $M_s$  is the sample weight before  
137 oil absorption,  $\rho_o$  is the density of the oil.

#### 138 **X-ray computed microtomography ( $\mu\text{CT}$ ) analysis**

139 Samples of approximately 5 by 5 mm were cut and analyzed using a SkyScan 1172  $\mu\text{CT}$  scanner (Bruker  
140 Corporation, Antwerp, Belgium). The samples were imaged at an isotropic voxel resolution of 5  $\mu\text{m}$ . the 3D  
141 imaging was done by rotating the object through 180° in steps of 0.4°, recording the projection images

142 using a cone beam configuration. 10 images were averaged for each position. A total of 1034 cross-section  
143 images per sample were generated with each sample requiring an acquisition time of about 1.2 h.

#### 144 **Spectroscopic analysis of samples**

145 NIR spectroscopy of the surface of the dosage forms was conducted on a BOMEM MB-160 spectrometer  
146 (ABB, Zürich, Switzerland), controlled by Horizon MB software version 3.2.5.2 (ABB). 32 scans were  
147 obtained for each spectrum covering the range from 4000 to 12000  $\text{cm}^{-1}$  and using a resolution of 8  $\text{cm}^{-1}$ . A  
148 spectralon reference standard (LabSphere Inc, North Sutton, NH, USA) was used to obtain a reference  
149 measurement before analyzing the samples. The samples were placed with each printed square centered  
150 on top of the analysis window.

151 Near-infrared transmission (tNIR) spectroscopy was conducted on the BOMEM MB-160 equipped with a  
152 tablet sampler (Tablet Samplir, ABB). The sampler has 4 signal enhancement levels, depending on the  
153 opaqueness of the samples. The level was kept at 1 (lowest) throughout the measurements. 64 scans were  
154 obtained for each spectrum covering 5800 to 12000  $\text{cm}^{-1}$  at a resolution of 8  $\text{cm}^{-1}$ . A transmission  
155 spectralon was used to obtain the reference measurement. The spectralon was kept in place while  
156 measuring the samples in order to decrease signal intensity and avoid oversaturating the detector.

157 Raman spectroscopy in a backscattering setup was done on a Kaiser RXN1 Microprobe (Kaiser Optical  
158 Systems, Ann Arbor, MI, USA) with a PhaT-probe (Kaiser Optical Systems), controlled by HoloGRAMS  
159 software v 4.1. The laser wavelength used was 785 nm and the Raman shift from 150 to 1900  $\text{cm}^{-1}$  was  
160 measured, each spectrum comprising 5801 data points. The laser spot size was 6 mm on the center of the  
161 printed samples and 6 exposures of 10 s each were averaged for each sample, giving a total exposure time  
162 of 60 s. A transmission setup was used as described earlier (21). The excitation fiber was placed directly  
163 beneath the sample and the Raman scattered light was collected by the PhaT-probe. The power of the laser  
164 was 200 mW at the output of the fiber. 6 exposures of 5 s each were averaged, giving a total exposure time  
165 of 30 s.

#### 166 **Multivariate data analysis**

167 Spectral data (NIR and Raman) were analyzed using MatLab R2015a (Mathworks, Natick, MA, USA) and PLS  
168 Toolbox 8.0.1 (Eigenvector Research Inc, Manson, WA, USA). All the data were subjected to preprocessing  
169 in the form of Standard Normal Variate (SNV) transformation followed by Savitzky-Golay smoothing.  
170 Different window sizes were used, but all data were fitted to a 2<sup>nd</sup> order polynomial of which the 2<sup>nd</sup>  
171 derivative was taken. The regions in the spectra with the most contribution from the API were selected.

172 After preprocessing and spectral selection, the data were modeled using Partial Least Squares (PLS)  
173 regression. The samples were randomly split into a calibration and validation set, the calibration set  
174 containing 2/3 of the samples and the validation set 1/3. Cross-validation was done using venetian blinds  
175 with 6 splits and 1 sample per blind.

## 176 **Results and Discussion**

### 177 **Ink formulation and substrate preparation**

178 In this study, three different APIs, propranolol hydrochloride (propranolol), montelukast sodium  
179 (montelukast) and haloperidol were inkjet-printed on the porous sponge-like HPMC substrate. The  
180 substrate was developed as previously reported by our group to possess good absorption properties for the  
181 ink and good mechanical properties, i.e., to be flexible but at the same time, mechanically strong (22). The  
182 substrate was prepared from high-molecular weight hypromellose, containing various excipients: glycerol  
183 and macrogol 4000 were added as plasticizers, whereas polysorbate 20 was used as a surfactant, foaming  
184 agent and plasticizer. The resulting substrate was porous and the morphology of the surface pores  
185 reflected the shape of sublimated ice crystals. It is well-known that the shape and size of the formed ice  
186 crystals within the sample during freeze drying cycle would affect the microstructure and surface  
187 topography of the dried sample (23).

188 The formulation of haloperidol- (15) and propranolol-containing (22) inks were done according to  
189 previously published work. The montelukast ink was formulated based on the solubility of the API in  
190 ethanol, and then modified so that the rheological and surface tension characteristics of the ink would be  
191 suitable for printing. In order to maximize the possible printed dose, the printable solvent system with a  
192 high concentration of the drug in it was selected, i.e., 200 mg/ml of montelukast in 3:7 PG:Ethanol ratio.  
193 The viscosity and surface tension of the drug-containing inks were all within the printable range (Table II).

194 Table II. Surface tension and dynamic viscosity of the printed API-containing inks.

Ink	Surface Tension, mN/m	Viscosity, mPa·s
Propranolol	47.95 ± 0.12	3.11 ± 0.02
Montelukast	27.81 ± 0.15	10.95 ± 0.87
Haloperidol	28.34 ± 0.01	3.87 ± 0.05

195



196 The use of porous substrates made of water-soluble polymer (HPMC) enables printing of multiple layers of  
197 the ink on the dosage forms without the risk of smearing of the ink during subsequent handling and  
198 thereby uncontrolled loss of the API. Indeed, the ink could absorb into the pores of the substrate. The  
199 choice of HPMC as the polymer was based on it being water soluble, pharmaceutically approved and being  
200 compatible with APIs and the most common ink solvents, used for dissolving/suspending the APIs. It is  
201 known that HPMC is slightly soluble by ethanol. However, it was expected that the ethanol-based ink,  
202 jetted in picoliters during IJP, would rather absorb into the porous substrate than dissolve it. In contrast, it  
203 was expected that printing a water-based ink would dissolve the surface of the substrate. The substrates  
204 could easily be handled after printing and there was visually observed no smearing of the ink for any of the  
205 APIs (Fig. 1).

206 The behavior of each ink in contact with the substrate was dependent on whether the ink was ethanol- or  
207 water-based (Fig. 2). The ethanol-based ink containing montelukast dissolved the surface of the substrate  
208 slightly when a high number of layers were printed, due to HPMC being soluble in ethanol to some extent  
209 (Fig. 2B). The haloperidol ink contained ethanol and also lactic acid, which has been shown to dissolve  
210 HPMC (24). However, the ink was only printed in a maximum of 14 layers which did not appear to change  
211 the surface significantly. A slight dissolution of the surface was observed when analyzed by microscopy (Fig  
212 2C). The water-based ink containing propranolol dissolved the surface of the substrate (HPMC  
213 dissolves/hydrates in water,) and a continuous polymer film (skin) was formed on the surface of the  
214 substrate, which was particularly evident when a higher number of layers was printed. The porous nature  
215 of the substrates enabled them to quickly absorb the ethanol-based inks during printing without  
216 significant dissolution of the surface of the substrate, highlighting the suitability of HPMC-based porous  
217 substrates for IJP of ethanol-based inks.

218 Despite a slight surface dissolution of the substrate by montelukast inks with 30 layers printed (Fig 3B), the  
219 ethanol-based inks penetrated into the porous substrate as expected (Fig 3B and 3C). In general, the  
220 penetration depth of the ink was dependent on the localized variation of the density of the polymer within  
221 the substrate, i.e., the ink penetrated deeper into areas with larger pores compared to areas with smaller  
222 pores as shown in Fig 3B. The penetration depth, evaluated by optical microscopy varied between 500-600  
223  $\mu\text{m}$  for the montelukast ink, but it was difficult to assess the penetration depth for the haloperidol ink due  
224 to its weak color and the low amount of layers printed. The water-based propranolol ink concentrated at  
225 the surface of the substrate without penetration into pores of the carrier and propranolol was crystallized  
226 at the surface (Fig. 3D).

227 **Porosity and microstructure**

228 The new substrate was designed to be porous to facilitate the absorption of ink, and therefore the porosity  
229 of the carrier is a crucial parameter. Two different methods were investigated, a custom-developed oil  
230 absorption method and  $\mu$ CT for assessing the porosity of the substrate. While the oil absorption method  
231 only gives a single value of the porosity for a given type of substrate,  $\mu$ -CT is able to visualize the internal  
232 structure of the samples, e.g., the pore size, their distribution and potential anisotropy of the material (25).  
233 The porosity of the freeze-dried substrate as measured by the oil absorption method was  $0.92 \pm 0.02$  ( $n =$   
234  $3$ ), showing that the freeze dried foams were highly porous as expected. The apparent porosity of the  
235 samples was lower for the  $\mu$ CT measurements, which gave values between 0.7-0.8 (results not shown). This  
236 is likely due to the different nature of the techniques with  $\mu$ CT being non-destructive and non-invasive  
237 compared to oil absorption, where interactions between the oil and the substrate take place. Despite the  
238 differences, both methods indicate that highly porous substrates were prepared using the freeze-drying  
239 method. The internal structure of the samples as measured by  $\mu$ CT revealed pores of varying size,  
240 presumably due to the variation in the size of the ice crystals during the freeze drying process. Two similarly  
241 prepared substrates had different apparent pore sizes (Fig. 4). This difference could be due to internal  
242 variation within the freeze drier during the freeze drying process, e.g., in one part of the freeze drier the  
243 formation of ice crystals is faster, resulting in small ice crystals and thereby in small pores, while in another  
244 part the nucleation is slower resulting in larger crystals and thereby larger pores. It could also be due to the  
245 preparation method: variation in the distribution of the components within the formulation could affect  
246 the morphology of the product after freeze-drying. Better control of the freeze drying process parameters  
247 to affect the formation of ice crystals might alleviate this (23). Despite the observed differences in the  
248 porosity level and pore size, the samples appeared relatively homogenous.

#### 249 **Drug content of the printed dosage forms**

250 In this study, the doses selected for printing of montelukast and haloperidol reflect clinical doses, i.e.,  
251 haloperidol has a recommended daily dose between 0.5 and 4 mg for treatment of first-episode psychosis  
252 (26) and between 1 and 4 mg for treatment of schizophrenia (27). For montelukast the range of clinically  
253 available doses is 4- 10 mg (28). For propranolol, the doses correspond to the daily uptake for treatment of  
254 infantile haemangioma (29), but higher doses are required in other indications (30). Despite not achieving  
255 the doses for adult treatment, they were printed in order to assess the effects of a water-based ink on the  
256 new substrates and the suitability for spectroscopic analysis.

257 The amount of the API deposited per layer is dependent on the concentration of API in the ink formulation,  
258 the droplet size and the area printed. For all three APIs, the content measured by HPLC correlated linearly  
259 with the amount of layers printed, indicating that the printing parameters did not vary significantly during

260 the process (Figure 5). There was a slight deviation from the calculated content, but it was deemed  
261 acceptable.

## 262 **Spectral analysis**

### 263 *Development of models*

264 The Raman and NIR spectra contain both physical and chemical information and must therefore be treated  
265 using preprocessing algorithms prior to modeling. In this study the focus was on the quantitative analysis of  
266 the API, therefore spectral variation from physical effects, such as density variation and pore size of the  
267 substrate, had to be addressed. This was done by systematic pretreatment of all the data. The optimal  
268 window size, polynomial fitting and derivative applied is highly dependent on the nature of each analysis  
269 (31), therefore various approaches for the optimization of the Savitzky-Golay preprocessing were  
270 attempted and combinations of different preprocessing parameters were used as described in Table III.

271 The substrate had a weak Raman scattering signal, likely a combination of two factors: (i) the primary  
272 ingredient of the substrate being HPMC which itself shows weak Raman scattering, and (ii) the low density  
273 of the substrate due to the sponge-like structure weakens the Raman signal. This makes the developed  
274 HPMC-based highly porous wafers good candidates for non-destructive analysis of printing APIs by Raman  
275 spectroscopy. Montelukast proved to have a strong Raman scattering signal in both transmission and  
276 backscatter modes. Haloperidol had a weak signal in backscatter mode, but little to no signal in  
277 transmission mode. Analysis of the propranolol-containing samples was complicated by the presence of  
278 brilliant blue in the ink, which induced fluorescence, making Raman spectroscopy unsuitable for analysis of  
279 the propranolol containing samples. The montelukast samples, both measured in transmission and  
280 backscatter modes, contained a strong Raman contribution from montelukast in the region from 700-1700  
281  $\text{cm}^{-1}$ . For haloperidol, the signal contribution was very low in transmission mode, however the signal was  
282 strong enough in backscatter mode to achieve a good signal useful for modeling.

283 Raman spectroscopy has limitations in regard to quantitative analysis of inkjet printed pharmaceuticals. For  
284 instance, the presence of fluorescence is a disturbing factor, which can arise from the substrate, the API or  
285 the ink constituents. In addition, different compounds can possess varying Raman scattering abilities that  
286 have to be taken into account when selection the ink-substrate combination. For example, if the substrate  
287 has a strong Raman contribution while the API has a weak contribution, determining the printed API  
288 content from the Raman spectrum can be challenging. Furthermore, when using a backscatter Raman  
289 setup, the penetration depth of the laser is of paramount importance; If the printed ink has penetrated  
290 deeper than the Raman laser, an incorrect API content may be predicted by the analysis.

291 All the samples were analyzed by transmission NIR (tNIR). While all the APIs have strong and characteristic  
 292 spectra when measured as pure powders, very little signal could be obtained when printed on the  
 293 substrate. The obtained PLS models contained a large number of LVs and had poor prediction power. This  
 294 technique was therefore discarded for quantitative analysis of the printed dosage forms. There can be  
 295 multiple reasons for the failure of tNIR in analyzing the printed dosage forms. First of all, the used tNIR  
 296 setup was optimized for tablet samples. Secondly, the used substrates are porous and require the  
 297 reduction of the signal strength in order to not oversaturate the detector. This may cause the reduction in  
 298 the spectral contribution from the APIs. Due to the failure of tNIR, where the entire bulk of the printed  
 299 dosage form was analyzed, focus was then switched to surface NIR. Here, only the surface of the printed  
 300 dosage forms was analyzed. All the APIs had strong signal contributions from the drug-printed samples  
 301 compared to the blank substrates. Therefore, the resulting spectra contained contributions from both the  
 302 substrate and the studied API (supplementary material). The PLS models for surface NIR showed excellent  
 303 predictive performance for both montelukast and propranolol, while haloperidol showed worse predictive  
 304 performance.

305 The quality of the PLS models for the prediction of the API content was assessed by evaluating the number  
 306 of latent variables (LVs), the relative contribution of each LV, the root-mean square error of cross-  
 307 validation/prediction (RMSECV and RMSEP, respectively) and the R<sup>2</sup>-value of cross-validation and  
 308 prediction. The number of latent variables gives information on the complexity of the model and it should  
 309 be evaluated against the RMSECV and RMSEP values that should be as small as possible. The optimal  
 310 selection of LVs gives usually a relatively low RMSECV and RMSEP and using a higher number of LVs does  
 311 not improve predictive power of the model. The resulting selection of LVs should also ideally give a high R<sup>2</sup>  
 312 value for both cross-validation and prediction, indicating that the predicted content gives a value close to  
 313 the content measured by HPLC. The optimal selected models for all APIs and techniques are gathered in  
 314 Table III.

315 Table III. Summary of working PLS models for the different methods and APIs.

API/substrate	Method	Variable selection, cm <sup>-1</sup>	Preprocessing, (window size, polynomial, derivative)	LVs	RMSECV/RMSEP, mg	R <sup>2</sup> CV/ R <sup>2</sup> P
Montelukast	Transmission Raman	150-1900	SNV, SG (81, 2, 2)	3	0.39/0.42	0.99/0.99

	Surface Raman	150-1900	SNV, SG (81,2,2)	4	0.81/0.86	0.96/0.97
	Surface NIR	3800-6400	SNV, SG (15, 2, 2)	4	0.34/0.39	0.99/0.99
Haloperidol	Surface Raman	150-1900	SG (51, 2, 2)	2	0.15/0.15	0.98/0.99
	Surface NIR	4100-6600	SNV, SG 31,2,2	5	0.24/0.20	0.96/0.97
Propranolol	Surface NIR	4100-6300	SNV, SG 31 2 2	5	0.21/0.23	0.98/0.99

316 RMSECV: root mean square error of cross-validation, RMSEP: root mean square error of prediction, R<sup>2</sup> CV:  
317 correlation coefficient of cross-validation sets, R<sup>2</sup> P: correlation coefficient of validation set

318 Table IV. Summary of the applicability of the spectroscopic methods for the non-destructive quantification  
319 of different APIs printed on porous substrates.

	Montelukast	Haloperidol	Propranolol
Transmission Raman	Yes	No	No
Surface Raman	Yes	Yes	No
Transmission NIR	No	No	No
Surface NIR	Yes	Yes	Yes

320

321 Comparing the different spectroscopic techniques and models studied does not yield an ideal method for  
322 all cases (Table IV). The appropriate analytical and modeling methods are API-dependent, and are affected  
323 by the resulting ink formulation and the substrate. Montelukast, being a strong Raman scatterer, gave good  
324 results when using the transmission setup, however poor fitting was achieved for the surface Raman setup.  
325 The good fitting for the transmission model can be ascribed to the transmission setup measuring the entire  
326 bulk of the dosage form, independent of penetration depth of the ink or variation in the density of the  
327 porous substrate. Surface Raman spectroscopy had a poorer performance, which can be due to the Raman  
328 laser not penetrating deep enough to get a linear correlation between the number of layers printed and the  
329 resulting spectra. Furthermore, both methods required a combination of SNV and Savitzky-Golay

330 smoothing algorithm for preprocessing of the raw spectra. SNV, originally developed for standardization of  
331 NIR spectra, first transforms each spectrum to mean zero and unit standard deviation after which the  
332 Savitsky-Golay preprocessing smoothens the spectra and enhances shoulders and subtle differences in the  
333 spectra. While surface Raman yielded a poor model for montelukast, the surface NIR method yielded a  
334 good fitting, indicating that the penetration depth for surface NIR spectroscopy being high enough to  
335 measure the entire amount of the printed API. The best fitting for the predictive quantitative analysis of  
336 haloperidol was shown by the surface Raman method, compared to the NIR method. Interestingly, the best  
337 fit was achieved when using only Savitzky-Golay preprocessing without SNV transformation. The reason for  
338 this can be found in the raw spectra, which contained weak contributions from haloperidol and the  
339 substrate, plus a strong background contribution inherent to the instrument. Applying SNV means that the  
340 background contribution would be enhanced in the SG processed spectra, thereby weakening the  
341 prediction ability of the model (supplementary material). For propranolol, due to the addition of brilliant  
342 blue to the ink, it induced fluorescence with Raman spectroscopy, only NIR was usable, where a good  
343 predictability of the API content was achieved.

## 344 **Conclusion**

345 The use of spectroscopic techniques made it possible to fast and accurately determine the API content in  
346 dosage forms prepared by inkjet printing on porous substrates suitable for the printing process. Selecting a  
347 single optimal method for non-destructive determination of the API content in any printed sample is close  
348 to impossible as each technique has its advantages and drawbacks. That said, Raman in both transmission  
349 and reflectance mode and NIR spectroscopy in surface mode could be used in combination, complementing  
350 each other. However, in specific cases when one method fails for a given API (e.g. Raman spectroscopy due  
351 to fluorescence), another technique (NIR spectroscopy) could be used alone for assessing the API content.

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